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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : <b>A61K 45/06, A61P 9/00</b>	<b>A1</b>	(11) International Publication Number: <b>WO 00/38722</b>
		(43) International Publication Date: <b>6 July 2000 (06.07.00)</b>

(21) International Application Number: <b>PCT/US99/27943</b>	(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
(22) International Filing Date: <b>17 December 1999 (17.12.99)</b>	
(30) Priority Data: 60/113,955 23 December 1998 (23.12.98) US 60/142,722 7 July 1999 (07.07.99) US	
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(54) Title: **COMBINATIONS OF CHOLESTERYL ESTER TRANSFER PROTEIN INHIBITORS AND HMG CoA REDUCTASE INHIBITORS FOR CARDIOVASCULAR INDICATIONS**

(57) Abstract

The present invention provides combinations of cardiovascular therapeutic compounds for the prophylaxis or treatment of cardiovascular disease including hypercholesterolemia, atherosclerosis, or hyperlipidemia. Combinations disclosed include an HMG CoA reductase inhibitor combined with a cholestrylo ester transfer protein (CETP) inhibitor.

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-1-

**Combinations of Cholesteryl Ester Transfer Protein  
Inhibitors and HMG CoA Reductase Inhibitors for  
Cardiovascular Indications**

5 This application claims priority of U.S. provisional application Ser. No. 60/142,722 filed Jul. 7, 1999 and of U.S. provisional application Ser. No. 60/113,955 filed Dec. 23, 1998.

10

**BACKGROUND OF THE INVENTION**

**Field of the Invention**

The present invention relates to methods of treating cardiovascular diseases, and specifically relates to 15 combinations of compounds, compositions, and methods for their use in medicine, particularly in the prophylaxis and treatment of hyperlipidemic conditions such as are associated with atherosclerosis, hypercholesterolemia, and other coronary artery disease in mammals. More 20 particularly, the invention relates to cholesteryl ester transfer protein (CETP) activity inhibiting compounds. The invention also relates to HMG CoA reductase inhibitors (statins).

25 **Description of Related Art**

It is well-settled that hyperlipidemic conditions associated with elevated concentrations of total cholesterol and low-density lipoprotein (LDL) cholesterol are major risk factors for coronary heart 30 disease and particularly atherosclerosis. Numerous studies have demonstrated that a low plasma concentration of high density lipoprotein (HDL) cholesterol is a powerful risk factor for the development of atherosclerosis (Barter and Rye,

-2-

Atherosclerosis, 121, 1-12 (1996). HDL is one of the major classes of lipoproteins that function in the transport of lipids through the blood. The major lipids found associated with HDL include cholesterol, 5 cholestryl ester, triglycerides, phospholipids and fatty acids. The other classes of lipoproteins found in the blood are low density lipoprotein (LDL), intermediate density lipoprotein (IDL), and very low density lipoprotein (VLDL). Since low levels of HDL 10 cholesterol increase the risk of atherosclerosis, methods for elevating plasma HDL cholesterol would be therapeutically beneficial for the treatment of atherosclerosis and other diseases associated with accumulation of lipid in the blood vessels. These 15 diseases include, but are not limited to, coronary heart disease, peripheral vascular disease, and stroke.

Atherosclerosis underlies most coronary artery disease (CAD), a major cause of morbidity and mortality in modern society. High LDL cholesterol (above about 180 20 mg/dl) and low HDL cholesterol (below 35 mg/dl) have been shown to be important contributors to the development of atherosclerosis. Other diseases or risk factors, such as peripheral vascular disease, stroke, and hypercholesterolaemia are negatively affected by adverse 25 HDL/LDL ratios.

Interfering with the recirculation of bile acids from the lumen of the intestinal tract is found to reduce the levels of serum cholesterol in a causal relationship. Epidemiological data has accumulated which indicates such 30 reduction leads to an improvement in the disease state of atherosclerosis. Stedronsky, in "Interaction of bile acids and cholesterol with nonsystemic agents having hypcholesterolemic properties," Biochimica et Biophysica

-3-

Acta, 1210, 255-287 (1994) discusses the biochemistry, physiology and known active agents surrounding bile acids and cholesterol.

Inhibition of cholesteryl ester transfer protein 5 (CETP) has been shown to effectively modify plasma HDL/LDL ratios, and is expected to check the progress and/or formation of certain cardiovascular diseases. CETP is a plasma protein that facilitates the movement 10 of cholesteryl esters and triglycerides between the various lipoproteins in the blood (Tall, J. Lipid Res., 34, 1255-74 (1993)). The movement of cholesteryl ester from HDL to LDL by CETP has the effect of lowering HDL cholesterol. It therefore follows that inhibition of CETP should lead to elevation of plasma HDL cholesterol 15 and lowering of plasma LDL cholesterol, thereby providing a therapeutically beneficial plasma lipid profile. Evidence of this effect is described in McCarthy, Medicinal Res. Revs., 13, 139-59 (1993). Further evidence of this effect is described in Sitori, 20 Pharmac. Ther., 67, 443-47 (1995)). This phenomenon was first demonstrated by Swenson et al., (J. Biol. Chem., 264, 14318 (1989)) with the use of a monoclonal antibody that specifically inhibits CETP. In rabbits, the antibody caused an elevation of the plasma HDL 25 cholesterol and a decrease in LDL cholesterol. Son et al. (Biochim. Biophys. Acta, 795, 743-480 (1984)) describe proteins from human plasma that inhibit CETP. U.S. Patent 5,519,001, herein incorporated by reference, issued to Kushwaha et al., describes a 36 amino acid 30 peptide derived from baboon apo C-1 that inhibits CETP activity. Cho et al. (Biochim. Biophys. Acta 1391, 133-144 (1998)) describe a peptide from hog plasma that inhibits human CETP. Bonin et al. (J. Peptide Res., 51,

-4-

216-225 (1998)) disclose a decapeptide inhibitor of CETP. A depsipeptide fungal metabolite is disclosed as a CETP inhibitor by Hedge et al. in *Bioorg. Med. Chem. Lett.*, 8, 1277-80 (1998).

- 5 There have been several reports of non-peptidic compounds that act as CETP inhibitors. Barrett et al. (J. Am. Chem. Soc., 118, 7863-63 (1996)) describe cyclopropane-containing CETP inhibitors. Further cyclopropane-containing CETP inhibitors are described by
- 10 Kuo et al. (J. Am. Chem. Soc., 117, 10629-34 (1995)). Pietzonka et al. (Bioorg. Med. Chem. Lett., 6, 1951-54 (1996)) describe phosphonate-containing analogs of cholesteryl ester as CETP inhibitors. Coval et al. (Bioorg. Med. Chem. Lett., 5, 605-610 (1995)) describe
- 15 Wiedendiol-A and -B, and related sesquiterpene compounds as CETP inhibitors. Lee et al. (J. Antibiotics, 49, 693-96 (1996)) describe CETP inhibitors derived from an insect fungus. Busch et al. (Lipids, 25, 216-220, (1990)) describe cholesteryl acetyl bromide as a CETP
- 20 inhibitor. Morton and Zilversmit (J. Lipid Res., 35, 836-47 (1982)) describe that p-chloromercuriphenyl sulfonate, p-hydroxymercuribenzoate and ethyl mercurithiosalicylate inhibit CETP. Connolly et al. (Biochem. Biophys. Res. Comm., 223, 42-47 (1996))
- 25 describe other cysteine modification reagents as CETP inhibitors. Xia et al. describe 1,3,5-triazines as CETP inhibitors (Bioorg. Med. Chem. Lett., 6, 919-22 (1996)). Bisgaier et al. (Lipids, 29, 811-8 (1994)) describe 4-phenyl-5-tridecyl-4H-1,2,4-triazole-thiol as
- 30 a CETP inhibitor. Additional triazole CETP inhibitors are described in U.S. Patent Application Serial No. 09/153,360, herein incorporated by reference. Sikorski

-5-

et al. disclosed further novel CETP inhibitors in PCT Patent Application No. WO 9914204.

Substituted 2-mercaptoaniline amide compounds can be used as CETP inhibitors and such therapeutic 5 compounds are described by H. Shinkai et al. in PCT Patent Application No. WO 98/35937.

Some substituted heteroalkylamine compounds are known as CETP inhibitors. In European Patent Application No. 796846, Schmidt et al. describe 2-aryl-10 substituted pyridines as cholesterol ester transfer protein inhibitors useful as cardiovascular agents. One substituent at C<sub>3</sub> of the pyridine ring can be an hydroxyalkyl group. In European Patent Application No. 801060, Dow and Wright describe heterocyclic derivatives 15 substituted with an aldehyde addition product of an alkylamine to afford 1-hydroxy-1-amines. These are reported to be β<sub>3</sub>-adrenergic receptor agonists useful for treating diabetes and other disorders. In Great Britain Patent Application No. 2305665, Fisher et al. 20 disclose 3-agonist secondary amino alcohol substituted pyridine derivatives useful for treating several disorders including cholesterol levels and atherosclerotic diseases. In European Patent Application No. 818448 (herein incorporated by reference), Schmidt et al. describe tetrahydroquinoline 25 derivatives as cholesterol ester transfer protein inhibitors. European Patent Application No. 818197, Schmek et al. describe pyridines with fused heterocycles as cholesterol ester transfer protein inhibitors. 30 Brandes et al. in German Patent Application No. 19627430 describe bicyclic condensed pyridine derivatives as cholesterol ester transfer protein inhibitors. In PCT Patent Application No. WO 9839299, Muller-Gliemann et

- 6 -

al. describe quinoline derivatives as cholesteryl ester transfer protein inhibitors.

Polycyclic compounds that are useful as CETP inhibitors are also disclosed by A. Oomura et al. in 5 Japanese Patent No. 10287662. For example, therapeutic compounds having the structures C-1 and C-8 were prepared by culturing *Penicillium spp.*

Cycloalkylpyridines useful as CETP inhibitors are disclosed by Schmidt et al. in European Patent No. EP 10 818448. For example, the therapeutic compound having the structure C-9 is disclosed as being particularly effective as a CETP inhibitor.

Substituted tetrahydronaphthalene compounds useful as CETP inhibitors are described in PCT Patent 15 Application No. WO 9914174. Specifically described in that disclosure as a useful CETP inhibitor is (8S)-3-cyclopentyl-1-(4-fluorophenyl)-2-[(S)-fluoro(4-trifluoromethylphenyl)methyl]-8-hydroxy-6-spiroclobutyl-5,6,7,8-tetrahydronaphthalene.

20 Some 4-heteroaryl-tetrahydroquinolines useful as CETP inhibitors are described in PCT Patent Application No. WO 9914215. For example, that disclosure describes 3-(4-trifluoromethylbenzoyl)-5,6,7,8-tetrahydroquinolin-5-one as a useful CETP inhibitor.

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-7-

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- Some 4-heteroaryl-tetrahydroquinolines useful as CETP inhibitors are described in PCT Patent Application No. WO 9914215. For example, that disclosure describes 3-(4-trifluoromethylbenzoyl)-5,6,7,8-tetrahydroquinolin-5-one as a useful CETP inhibitor.
- In another approach to the reduction of total cholesterol, use is made of the understanding that HMG CoA reductase catalyzes the rate-limiting step in the biosynthesis of cholesterol (The Pharmacological Basis of Therapeutics, 9th ed., J.G. Hardman and L.E. Limberd, ed., McGraw-Hill, Inc., New York, pp. 884-888 (1996)). HMG CoA reductase inhibitors (including the class of therapeutics commonly called "statins") reduce blood serum levels of LDL cholesterol by competitive inhibition of this biosynthetic step (M.S. Brown, et al., J. Biol. Chem., 253, 1121-28 (1978)). Several statins have been developed or commercialized throughout the world. Mevastatin was among the first of the statins to be developed and it is described in U.S. Patent No. 3,983,140. Lovastatin, another important HMG CoA reductase inhibitor, is described in U.S. patent no. 4,231,938. Simvastatin is described in U.S. patent no. 4,444,784. Each of these HMG CoA reductase inhibitors contains a six-membered lactone function which apparently mimics the structure of HMG CoA.

- 8 -

in competition for the reductase. The HMG CoA reductase inhibitor class of cholesterol-lowering drugs is further exemplified by a group of drugs which contain 2,4-dihydroxyheptanoic acid functionalities rather than the 5 lactone. One member of this group is pravastatin, described in U.S. patent no. 4,346,227. Another HMG CoA reductase inhibitor which contains a 2,4-dihydroxyheptanoic acid group is fluvastatin, described in U.S. patent no. 5,354,772. A further HMG CoA reductase 10 inhibitor is ZD-4522 (S-4522), described by Watanabe et al., Bioorg. Med. Chem., 5(2), 437-444 (1997). Warnings of side effects from use of HMG CoA reductase inhibitors include liver dysfunction, skeletal muscle myopathy, rhabdomyolysis, and acute renal failure. Some of these 15 effects are exacerbated when HMG CoA reductase inhibitors are combined with fibrates or nicotinic acid.

Some combination therapies for the treatment of cardiovascular disease have been described in the literature. Combinations of IBAT inhibitors with HMG CoA 20 reductase inhibitors useful for the treatment of cardiovascular disease are disclosed in U.S. Patent Application No. 09/037,308.

A combination therapy of fluvastatin and nericin is described by J. Sasaki et al. (Id.). Those researchers 25 conclude that the combination of fluvastatin with nericin "at a dose of 750 mg/day dose does not appear to augment or attenuate beneficial effects of fluvastatin."

L. Cashin-Hemphill et al. (J. Am. Med. Assoc., 264 30 (23), 3013-17 (1990)) describe beneficial effects of a combination therapy of colestipol and niacin on coronary atherosclerosis. The described effects include

-9-

nonprogression and regression in native coronary artery lesions.

A combination therapy of acipimox and simvastatin shows beneficial HDL effects in patients having high triglyceride levels (N. Hoogerbrugge et al., J. Internal Med., 241, 151-55 (1997)).

Sitostanol ester margarine and pravastatin combination therapy is described by H. Gylling et al. (J. Lipid Res., 37, 1776-85 (1996)). That therapy is reported to simultaneously inhibit cholesterol absorption and lower LDL cholesterol significantly in non-insulin-dependent diabetic men.

Brown et al. (New Eng. J. Med., 323 (19), 1289-1339 (1990)) describe a combination therapy of lovastatin and colestipol which reduces atherosclerotic lesion progression and increase lesion regression relative to lovastatin alone.

A combination therapy of an apoB secretion inhibitor with a CETP inhibitor was disclosed by Chang et al. in PCT Patent Application No. WO 9823593.

Buch et al. (PCT Patent Application No. WO 9911263) describe a combination therapy comprising amlodipine and a statin compound for treating subjects suffering from angina pectoris, atherosclerosis, combined hypertension and hyperlipidemia, and to treat symptoms of cardiac arrest. Buch et al. describe in PCT Patent Application No. WO 9911259 a combination therapy comprising amlodipine and atorvastatin.

Scott et al. (PCT Patent Application No. WO 9911260) describe a combination therapy comprising atorvastatin and an antihypertensive agent.

Dettmar and Gibson (UK Patent Application No. GB 2329334 A) claim a therapeutic composition useful for

-10-

reducing plasma low density lipoprotein and cholesterol levels, wherein the composition comprises an HMG CoA reductase inhibitor and a bile complexing agent.

The above references show continuing need to find  
5 safe, effective agents for the prophylaxis or treatment of cardiovascular diseases.

Summary of the Invention

To address the continuing need to find safe and  
10 effective agents for the prophylaxis and treatment of cardiovascular diseases, combination therapies of cardiovascular drugs are now reported.

Among its several embodiments, the present invention provides a combination therapy comprising the use of a  
15 first amount of an CETP inhibitor and a second amount of another cardiovascular therapeutic useful in the prophylaxis or treatment of hyperlipidemia, atherosclerosis, or hypercholesterolemia, wherein said first and second amounts together comprise an anti-  
20 hyperlipidemic condition effective amount, an anti-atherosclerotic condition effective amount, or an anti-hypercholesterolemic condition effective amount of the compounds. For example one of the many embodiments of the present invention is a combination therapy comprising  
25 therapeutic dosages of an CETP inhibitor and an HMG CoA reductase inhibitor.

A further embodiment of the instant invention comprises the use of any of the cardiovascular combination therapies described herein for the prophylaxis or  
30 treatment of hypercholesterolemia, atherosclerosis, or hyperlipidemia. Therefore, in one embodiment the present invention provides a method for the prophylaxis or treatment of a hyperlipidemic condition comprising

-11-

administering to a patient in need thereof a combination in unit dosage form wherein the combination comprises a first amount of an HMG CoA reductase inhibiting compound and a second amount of a CETP inhibiting compound wherein  
5 the first amount and the second amount together comprise an anti-hyperlipidemic condition effective amount of the compounds.

In another embodiment, the present invention provides a method for the prophylaxis or treatment of an  
10 atherosclerotic condition comprising administering to a patient in need thereof a combination in unit dosage form wherein the combination comprises a first amount of an HMG CoA reductase inhibiting compound and a second amount of a CETP inhibiting compound wherein the first amount and the  
15 second amount together comprise an anti-atherosclerotic condition effective amount of the compounds.

In still another embodiment, the present invention provides method for the prophylaxis or treatment of hypercholesterolemia comprising administering to a patient  
20 in need thereof a combination in unit dosage form wherein the combination comprises a first amount of an HMG CoA reductase inhibiting compound and a second amount of a CETP inhibiting compound wherein the first amount and the second amount together comprise an anti-  
25 hypercholesterolemic condition effective amount of the compounds.

Further scope of the applicability of the present invention will become apparent from the detailed description provided below. However, it should be  
30 understood that the following detailed description and examples, while indicating preferred embodiments of the invention, are given by way of illustration only since various changes and modifications within the spirit and

scope of the invention will become apparent to those skilled in the art from this detailed description.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

5

The following detailed description is provided to aid those skilled in the art in practicing the present invention. Even so, this detailed description should not be construed to unduly limit the present invention as 10 modifications and variations in the embodiments discussed herein can be made by those of ordinary skill in the art without departing from the spirit or scope of the present inventive discovery.

The contents of each of the references cited herein, 15 including the contents of the references cited within these primary references, are herein incorporated by reference in their entirety.

a. Definitions

20 The following definitions are provided in order to aid the reader in understanding the detailed description of the present invention:

As used herein the term "CETP inhibitor" or "CETP inhibiting compound" means any entity derived from 25 chemical or biological sources which inhibits cholesteryl ester transfer protein activity.

As used herein the term "statin" or the term "HMG CoA reductase inhibitor" or the term "HMG CoA reductase inhibiting compound" mean any entity derived from chemical 30 or biological sources which inhibits HMG CoA reductase activity.

"Combination therapy" means the administration of two or more therapeutic agents to treat a hyperlipidemic

condition, for example atherosclerosis and hypercholesterolemia. Such administration encompasses co-administration of these therapeutic agents in a substantially simultaneous manner, such as in a single 5 capsule having a fixed ratio of active ingredients or in multiple, separate capsules for each inhibitor agent. In addition, such administration also encompasses use of each type of therapeutic agent in a sequential manner. In either case, the treatment regimen will provide beneficial 10 effects of the drug combination in treating the hyperlipidemic condition.

The phrase "therapeutically effective" is intended to qualify the combined amount of inhibitors in the combination therapy. This combined amount will achieve 15 the goal of reducing or eliminating the hyperlipidemic condition.

"Therapeutic compound" means a compound useful in the prophylaxis or treatment of a hyperlipidemic condition, including atherosclerosis and hypercholesterolemia.

20

b. Combinations

The combinations of the present invention will have a number of uses. For example, through dosage adjustment 25 and medical monitoring, the individual dosages of the therapeutic compounds used in the combinations of the present invention will be lower than are typical for dosages of the therapeutic compounds when used in monotherapy. The dosage lowering will provide advantages 30 including reduction of side effects of the individual therapeutic compounds when compared to the monotherapy. In addition, fewer side effects of the combination therapy

-14-

compared with the monotherapies will lead to greater patient compliance with therapy regimens.

Another use of the present invention will be in combinations having complementary effects or complementary modes of action. For example, HMG CoA reductase inhibitors control blood serum cholesterol levels by inhibiting an enzyme which is important in the biosynthesis of cholesterol. In contrast, CETP inhibitors inhibit the movement of cholestryl esters and triglycerides between the various lipoproteins in the blood.

Some individual CETP inhibitor compounds useful in the present invention are separately described in the following individual patent applications, each of which is 15 individually herein incorporated by reference.

- R9. U.S. Patent Application Serial No. 60/101661.
- R10. U.S. Patent Application Serial No. 60/101711.
- R11. U.S. Patent Application Serial No. 60/101660.
- 20 R12. U.S. Patent Application Serial No. 60/101664.
- R13. U.S. Patent Application Serial No. 60/101668.
- R14. U.S. Patent Application Serial No. 60/101662.
- R15. U.S. Patent Application Serial No. 60/101663.
- R16. U.S. Patent Application Serial No. 60/101669.
- 25 R17. U.S. Patent Application Serial No. 60/101667.
- R18. U.S. Patent Application Serial No. 09/401,916.
- R19. U.S. Patent Application Serial No. 09/405,524.
- R20. U.S. Patent Application Serial No. 09/404,638.
- R21. U.S. Patent Application Serial No. 09/404,638.
- 30 R22. U.S. Patent Application Serial No. 09/400,915.
- R23. U.S. Patent No. 5,932,587.
- R24. U.S. Patent No. 5,925,645.

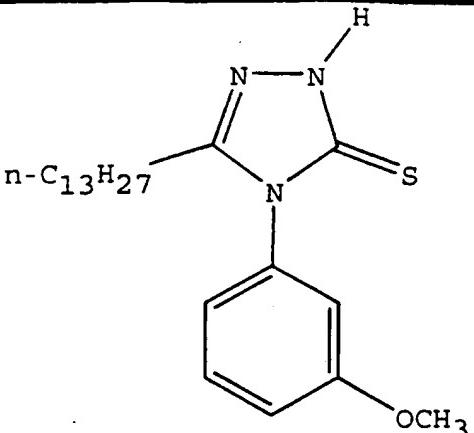
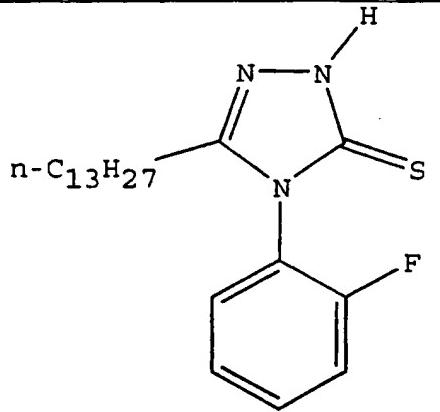
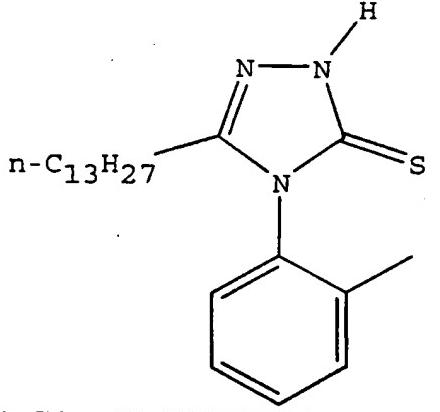
CETP inhibitor compounds of particular interest in the present invention include those shown in Table 1, as well as the diastereomers, enantiomers, racemates, salts, and tautomers of the CETP inhibitors of Table 1.

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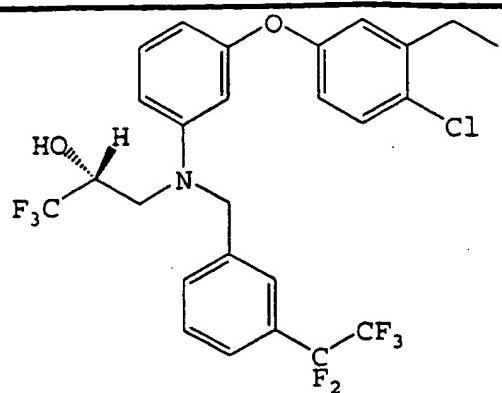
Table 1.

Compound Number	Structure
C-1	
C-2	
C-3	

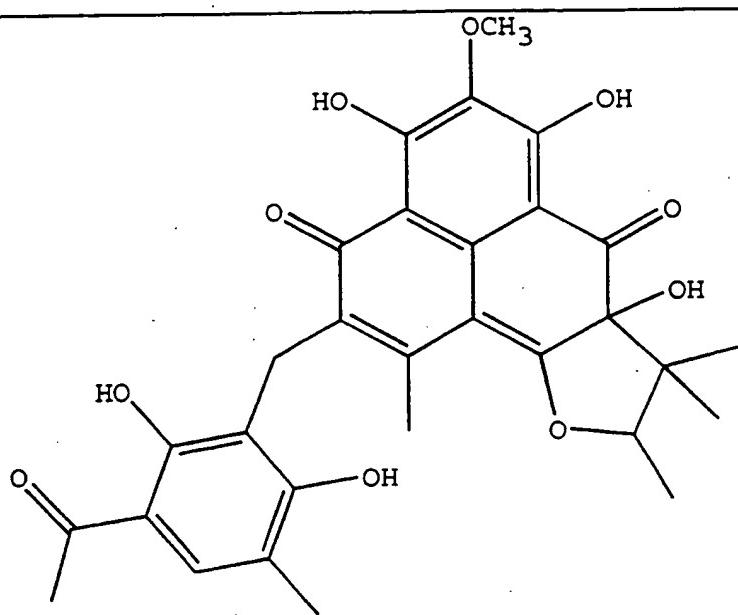
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C-4	
C-5	
C-6	
C-7	

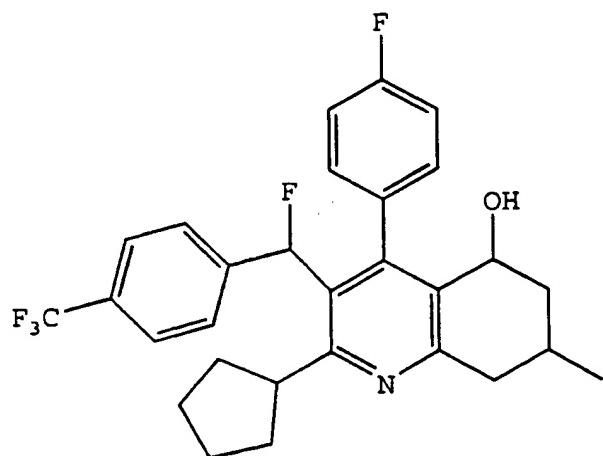
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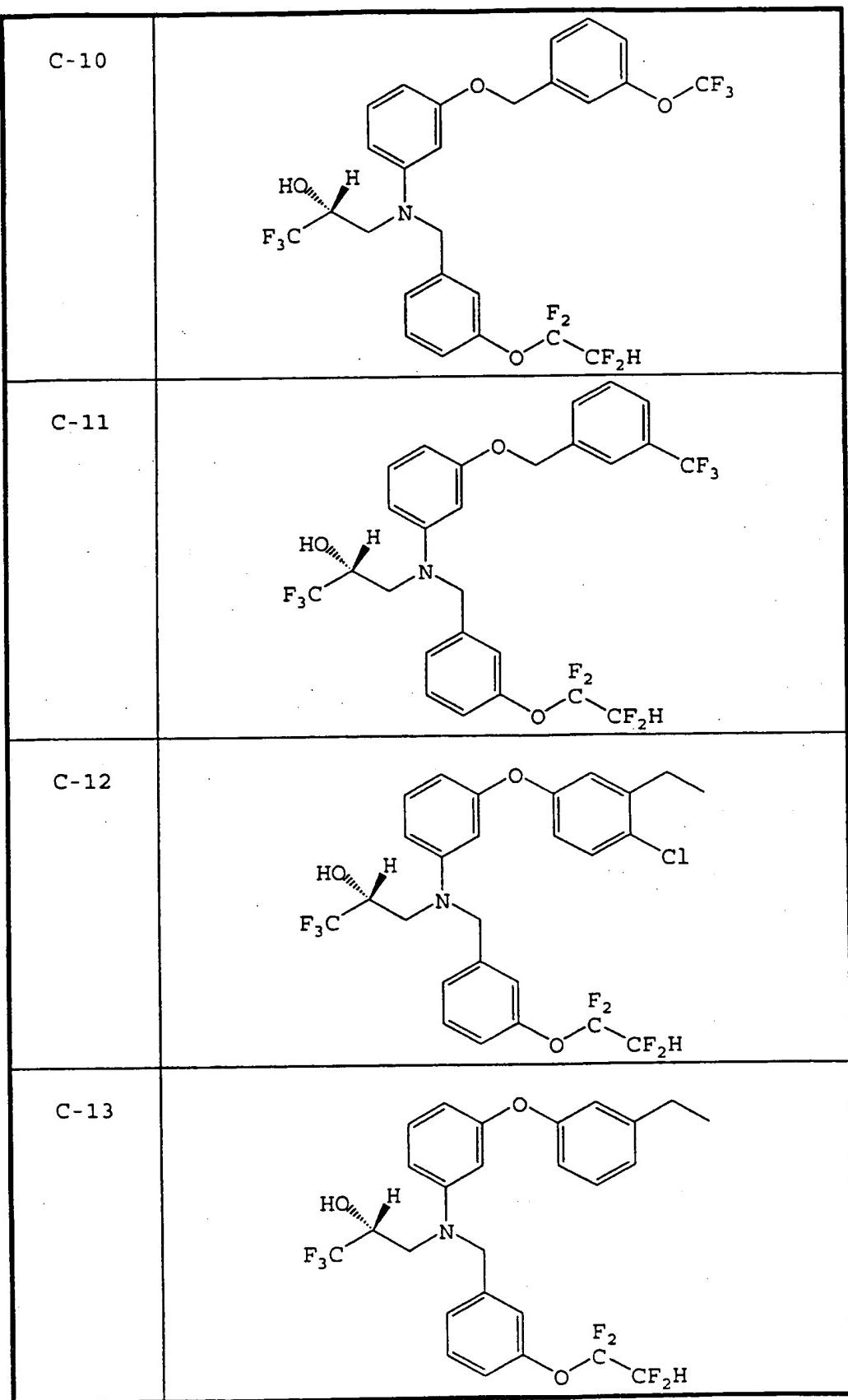
C-8



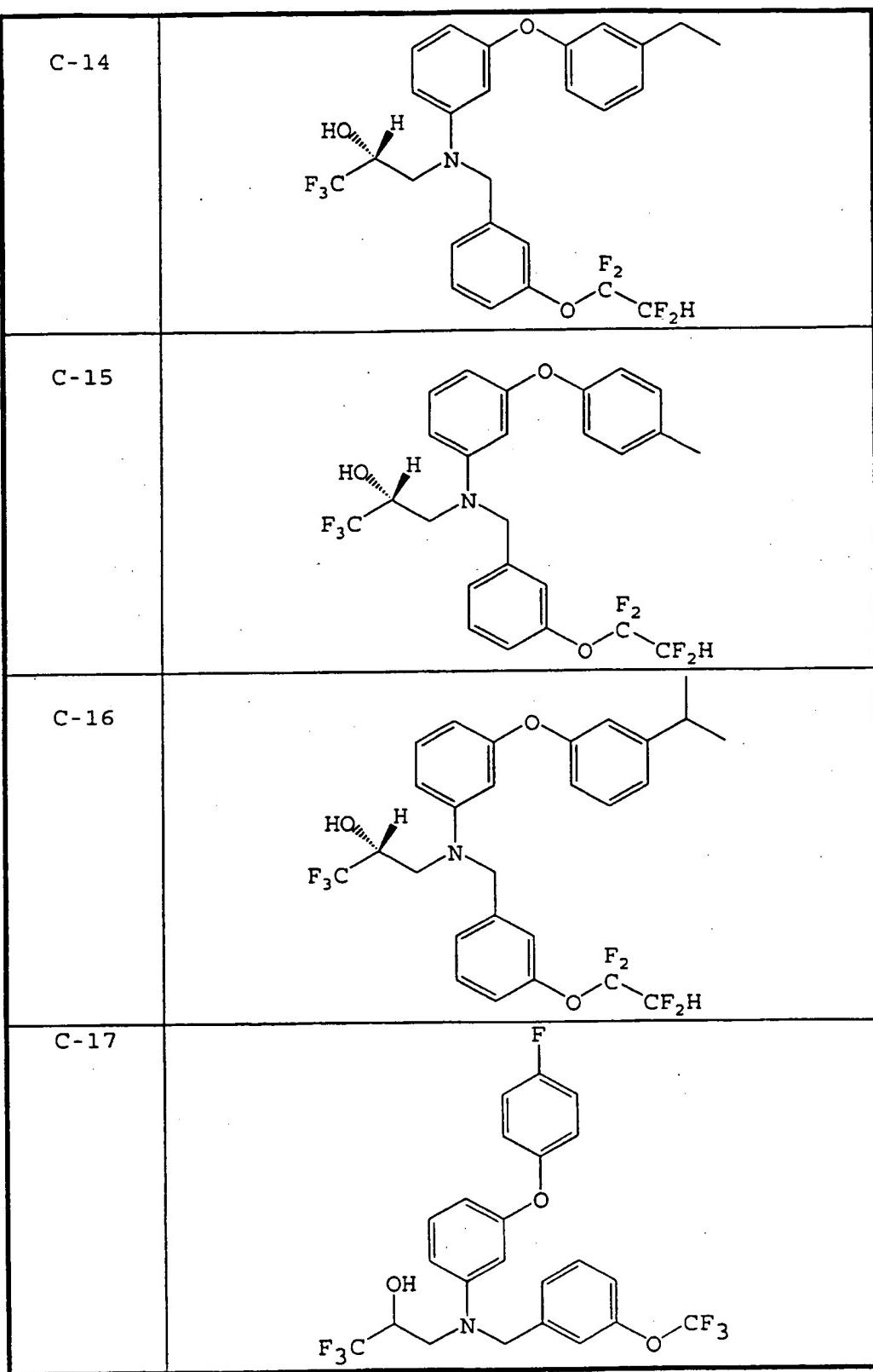
C-9

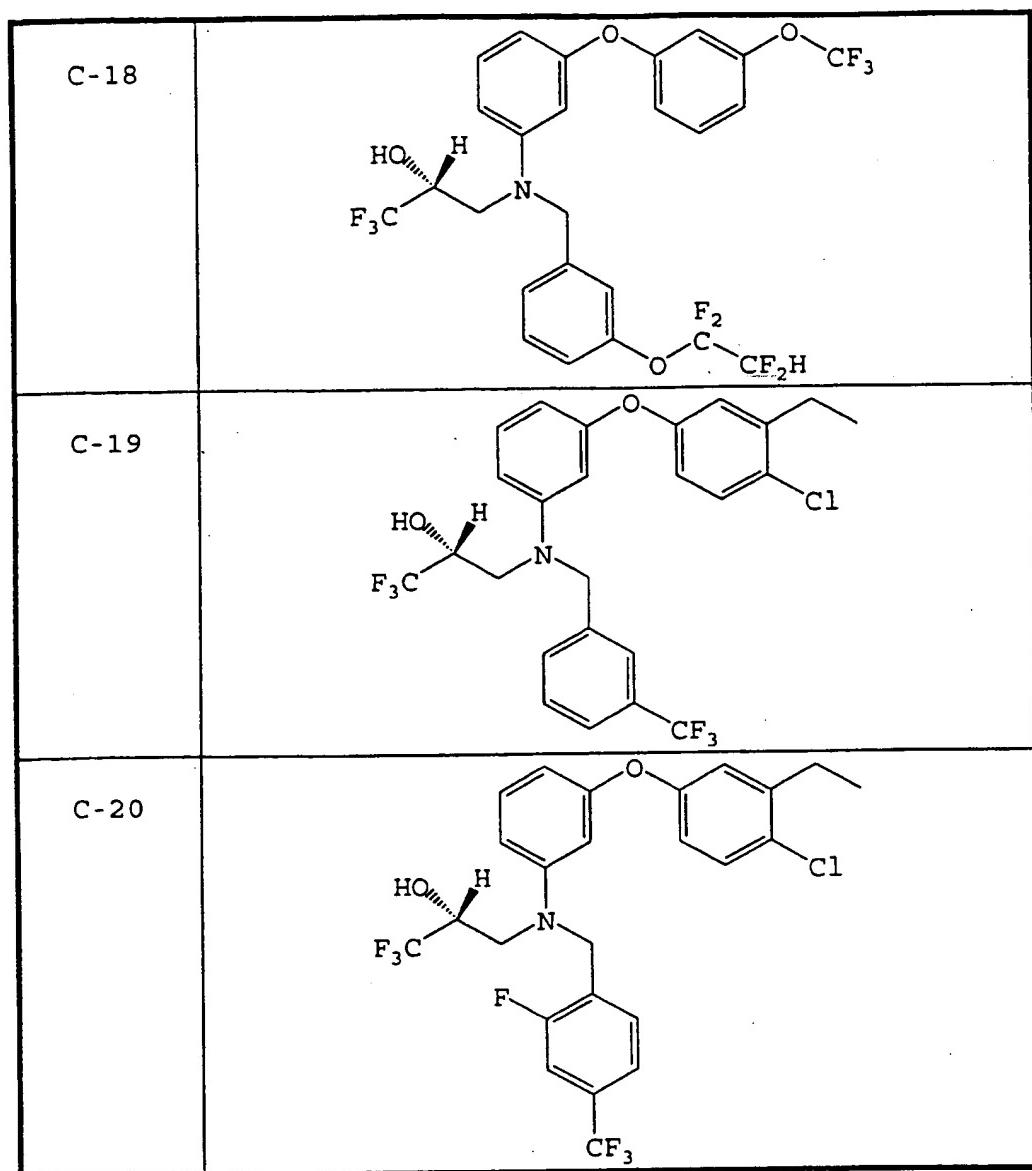


-18-



-19-





HMG CoA reductase inhibitors encompassing a wide range of structures are useful in the combinations and methods of the present invention. Some HMG CoA reductase inhibitors of particular interest in the present invention are described in Table 2. The therapeutic compounds of Table 2 can be used in the present invention in a variety of forms, including acid form, salt form, racemates, enantiomers, zwitterions, and tautomers. The individual

U.S. patents referenced in Table 2 are each herein incorporated by reference.

Table 2.

Compound Number	Common Name	CAS Registry Number	Patent Reference for Compound Per Se
G-113	Mevastatin	73573-88-3	U.S. 3,983,140
G-105	Lovastatin	75330-75-5	U.S. 4,231,938
G-201	Simvastatin	79902-63-9	U.S. 4,444,784
G-184	Pravastatin	81093-37-0	U.S. 4,346,227
G-75	Fluvastatin	93957-54-1	U.S. 4,739,073
G-14	Atorvastatin	134523-00-5	EP 409281

5

The compounds (for example, HMG CoA reductase inhibiting compounds or CETP inhibiting compounds) useful in the present invention can have no asymmetric carbon atoms, or, alternatively, the useful compounds can have one or more asymmetric carbon atoms. When the useful compounds have one or more asymmetric carbon atoms, they therefore include racemates and stereoisomers, such as diastereomers and enantiomers, in both pure form and in admixture. Such stereoisomers can be prepared using conventional techniques, either by reacting enantiomeric starting materials, or by separating isomers of compounds of the present invention.

Isomers may include geometric isomers, for example *cis*-isomers or *trans*-isomers across a double bond. All such isomers are contemplated among the compounds useful in the present invention.

The compounds useful in the present invention also include tautomers.

The compounds useful in the present invention as discussed below include their salts, solvates and prodrugs.

Dosages, Formulations, and Routes of Administration

The compositions of the present invention can be  
5 administered for the prophylaxis and treatment of  
hyperlipidemic diseases or conditions by any means,  
preferably oral, that produce contact of these compounds  
with their site of action in the body, for example in the  
ileum, the plasma, or the liver of a mammal, e.g., a  
10 human.

For the prophylaxis or treatment of the conditions  
referred to above, the compounds useful in the  
compositions and methods of the present invention can be  
used as the compound *per se*. Pharmaceutically acceptable  
15 salts are particularly suitable for medical applications  
because of their greater aqueous solubility relative to  
the parent compound. Such salts must clearly have a  
pharmaceutically acceptable anion or cation. Suitable  
pharmaceutically acceptable acid addition salts of the  
20 compounds of the present invention when possible include  
those derived from inorganic acids, such as hydrochloric,  
hydrobromic, phosphoric, metaphosphoric, nitric, sulfonic,  
and sulfuric acids, and organic acids such as acetic,  
benzenesulfonic, benzoic, citric, ethanesulfonic, fumaric,  
25 gluconic, glycolic, isothionic, lactic, lactobionic,  
maleic, malic, methanesulfonic, succinic, toluenesulfonic,  
tartaric, and trifluoroacetic acids. The chloride salt is  
particularly preferred for medical purposes. Suitable  
pharmaceutically acceptable base salts include ammonium  
30 salts, alkali metal salts such as sodium and potassium  
salts, and alkaline earth salts such as magnesium and  
calcium salts.

The anions useful in the present invention are, of course, also required to be pharmaceutically acceptable and are also selected from the above list.

The compounds useful in the present invention can be presented with an acceptable carrier in the form of a pharmaceutical composition. The carrier must, of course, be acceptable in the sense of being compatible with the other ingredients of the composition and must not be deleterious to the recipient. The carrier can be a solid or a liquid, or both, and is preferably formulated with the compound as a unit-dose composition, for example, a tablet, which can contain from 0.05% to 95% by weight of the active compound. Other pharmacologically active substances can also be present, including other compounds of the present invention. The pharmaceutical compositions of the invention can be prepared by any of the well known techniques of pharmacy, consisting essentially of admixing the components.

Optionally, the combination of the present invention can comprise a composition comprising an HMG CoA reductase inhibiting compound and a CETP inhibiting compound. In such a composition, the HMG CoA reductase inhibiting compound and the CETP inhibiting compound can be present in a single dosage form, for example a pill, a capsule, or a liquid which contains both of the compounds.

These compounds can be administered by any conventional means available for use in conjunction with pharmaceuticals, either as individual therapeutic compounds or as a combination of therapeutic compounds.

The amount of compound which is required to achieve the desired biological effect will, of course, depend on a number of factors such as the specific compound chosen, the use for which it is intended, the mode of

administration, and the clinical condition of the recipient.

A total daily dose of an HMG CoA reductase inhibitor can generally be in the range of from about 0.1 to about 5 100 mg/day in single or divided doses. Lovastatin, atorvastatin, or mevastatin, for example, generally are each administered separately in a daily dose of about 10 to about 80 mg/day. Fluvastatin is generally administered in a daily dose of about 20 to about 40 mg/day. 10 Cerivastatin is generally administered in a daily dose of about 0.1 to about 0.3 mg/day.

For a CETP inhibitor, a total daily dose of about 0.01 to about 100 mg/kg body weight/day, and preferably between about 0.5 to about 20 mg/kg body weight/day, may 15 generally be appropriate.

The daily doses described in the preceding paragraphs for the various therapeutic compounds can be administered to the patient in a single dose, or in proportionate multiple subdoses. Subdoses can be administered 2 to 6 20 times per day. Doses can be in sustained release form effective to obtain desired results.

In the case of pharmaceutically acceptable salts, the weights indicated above refer to the weight of the acid equivalent or the base equivalent of the therapeutic 25 compound derived from the salt.

Oral delivery of the combinations of the present invention can include formulations, as are well known in the art, to provide prolonged or sustained delivery of the drug to the gastrointestinal tract by any number of 30 mechanisms. These include, but are not limited to, pH sensitive release from the dosage form based on the changing pH of the small intestine, slow erosion of a tablet or capsule, retention in the stomach based on the

-25-

physical properties of the formulation, bioadhesion of the dosage form to the mucosal lining of the intestinal tract, or enzymatic release of the active drug from the dosage form. For some of the therapeutic compounds useful in the present invention (e.g., a CETP inhibitor or an HMG CoA reductase inhibitor), the intended effect is to extend the time period over which the active drug molecule is delivered to the site of action by manipulation of the dosage form.. Thus, enteric-coated and enteric-coated controlled release formulations are within the scope of the present invention. Suitable enteric coatings include cellulose acetate phthalate, polyvinylacetate phthalate, hydroxypropylmethylcellulose phthalate and anionic polymers of methacrylic acid and methacrylic acid methyl ester.

The combinations of the present invention can be delivered orally either in a solid, in a semi-solid, or in a liquid form. When in a liquid or in a semi-solid form, the combinations of the present invention can, for example, be in the form of a liquid, syrup, or contained in a gel capsule (e.g., a gel cap). In one embodiment, when a CETP inhibitor is used in a combination of the present invention, the CETP inhibitor can be provided in the form of a liquid, syrup, or contained in a gel capsule. In another embodiment, when an HMG CoA reductase inhibitor is used in a combination of the present invention, the HMG CoA reductase inhibitor can be provided in the form of a liquid, syrup, or contained in a gel capsule.

For a CETP inhibitor the intravenously administered dose can, for example, be in the range of from about 0.003 mg/kg body weight to about 1.0 mg/kg body weight, preferably from about 0.01 mg/kg body weight to about 0.75

mg/kg body weight, more preferably from about 0.1 mg/kg body weight to about 0.6 mg/kg body weight.

An HMG CoA reductase inhibitor can be intravenously administered, for example, be in the range of from about 5 0.03 mg/kg body weight to about 5.0 mg/kg body weight, preferably from about 0.1 mg/kg body weight to about 0.1 mg/kg body weight, more preferably from about 0.4 mg/kg body weight to about 0.6 mg/kg body weight.

The dose of any of these therapeutic compounds can be 10 conveniently administered as an infusion of from about 10 ng/kg body weight to about 100 ng/kg body weight per minute. Infusion fluids suitable for this purpose can contain, for example, from about 0.1 ng to about 10 mg, preferably from about 1 ng to about 10 mg per milliliter. 15 Unit doses can contain, for example, from about 1 mg to about 10 g of the compound of the present invention. Thus, ampoules for injection can contain, for example, from about 1 mg to about 100 mg.

Pharmaceutical compositions according to the present 20 invention include those suitable for oral, rectal, topical, buccal (e.g., sublingual), and parenteral (e.g., subcutaneous, intramuscular, intradermal, or intravenous) administration, although the most suitable route in any given case will depend on the nature and severity of the 25 condition being treated and on the nature of the particular compound which is being used. In most cases, the preferred route of administration is oral.

Pharmaceutical compositions suitable for oral administration can be presented in discrete units, such as 30 capsules, cachets, lozenges, or tablets, each containing a predetermined amount of at least one therapeutic compound useful in the present invention; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous

liquid; or as an oil-in-water or water-in-oil emulsion. As indicated, such compositions can be prepared by any suitable method of pharmacy which includes the step of bringing into association the active compound(s) and the carrier (which can constitute one or more accessory ingredients). In general, the compositions are prepared by uniformly and intimately admixing the active compound with a liquid or finely divided solid carrier, or both, and then, if necessary, shaping the product. For example, 10 a tablet can be prepared by compressing or molding a powder or granules of the compound, optionally with one or more accessory ingredients. Compressed tablets can be prepared by compressing, in a suitable machine, the compound in a free-flowing form, such as a powder or 15 granules optionally mixed with a binder, lubricant, inert diluent and/or surface active/dispersing agent(s). Molded tablets can be made by molding, in a suitable machine, the powdered compound moistened with an inert liquid diluent.

Pharmaceutical compositions suitable for buccal (sub-20 lingual) administration include lozenges comprising a compound of the present invention in a flavored base, usually sucrose, and acacia or tragacanth, and pastilles comprising the compound in an inert base such as gelatin and glycerin or sucrose and acacia.

25 Pharmaceutical compositions suitable for parenteral administration conveniently comprise sterile aqueous preparations of a compound of the present invention. These preparations are preferably administered intravenously, although administration can also be effected by means of 30 subcutaneous, intramuscular, or intradermal injection. Such preparations can conveniently be prepared by admixing the compound with water and rendering the resulting solution sterile and isotonic with the blood. Injectable

compositions according to the invention will generally contain from 0.1 to 5% w/w of a compound disclosed herein.

Pharmaceutical compositions suitable for rectal administration are preferably presented as unit-dose 5 suppositories. These can be prepared by admixing a compound of the present invention with one or more conventional solid carriers, for example, cocoa butter, and then shaping the resulting mixture.

Pharmaceutical compositions suitable for topical 10 application to the skin preferably take the form of an ointment, cream, lotion, paste, gel, spray, aerosol, or oil. Carriers which can be used include petroleum jelly (e.g., Vaseline), lanolin, polyethylene glycols, alcohols, and combinations of two or more thereof. The active 15 compound is generally present at a concentration of from 0.1 to 50% w/w of the composition, for example, from 0.5 to 2%.

Transdermal administration is also possible.

Pharmaceutical compositions suitable for transdermal 20 administration can be presented as discrete patches adapted to remain in intimate contact with the epidermis of the recipient for a prolonged period of time. Such patches suitably contain a compound of the present invention in an optionally buffered, aqueous solution, 25 dissolved and/or dispersed in an adhesive, or dispersed in a polymer. A suitable concentration of the active compound is about 1% to 35%, preferably about 3% to 15%. As one particular possibility, the compound can be delivered from the patch by electrotransport or 30 iontophoresis, for example, as described in Pharmaceutical Research, 3(6), 318 (1986).

In any case, the amount of active ingredient that can be combined with carrier materials to produce a single

dosage form to be administered will vary depending upon the host treated and the particular mode of administration.

The solid dosage forms for oral administration 5 including capsules, tablets, pills, powders, gel caps, and granules noted above comprise one or more compounds useful in the present invention admixed with at least one inert diluent such as sucrose, lactose, or starch. Such dosage forms may also comprise, as in normal practice, additional 10 substances other than inert diluents, e.g., lubricating agents such as magnesium stearate or solubilizing agents such as cyclodextrins. In the case of capsules, tablets, powders, granules, gel caps, and pills, the dosage forms may also comprise buffering agents. Tablets and pills can 15 additionally be prepared with enteric coatings.

Liquid dosage forms for oral administration can include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and elixirs containing inert diluents commonly used in the art, such as water. Such 20 compositions may also comprise adjuvants, such as wetting agents, emulsifying and suspending agents, and sweetening, flavoring, and perfuming agents.

Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions may be 25 formulated according to the known art using suitable dispersing or setting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a nontoxic parenterally acceptable diluent or solvent, for example, 30 as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally

-30-

employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of  
5 injectables.

Pharmaceutically acceptable carriers encompass all the foregoing and the like.

In combination therapy, administration of two or more of the therapeutic agents useful in the present invention  
10 may take place sequentially in separate formulations, or may be accomplished by simultaneous administration in a single formulation or separate formulations.

Administration may be accomplished by oral route, or by intravenous, intramuscular, or subcutaneous injections.

15 The formulation may be in the form of a bolus, or in the form of aqueous or non-aqueous isotonic sterile injection solutions or suspensions. These solutions and suspensions may be prepared from sterile powders or granules having one or more pharmaceutically-acceptable carriers or  
20 diluents, or a binder such as gelatin or hydroxypropylmethyl cellulose, together with one or more of a lubricant, preservative, surface active or dispersing agent.

For oral administration, the pharmaceutical  
25 composition may be in the form of, for example, a tablet, capsule, suspension, or liquid. Capsules, tablets, etc., can be prepared by conventional methods well known in the art. The pharmaceutical composition is preferably made in the form of a dosage unit containing a particular amount  
30 of the active ingredient or ingredients. Examples of dosage units are tablets or capsules. These may with advantage contain one or more therapeutic compound in an amount described above. For example, in the case of an

HMG Co-A reductase inhibitor, the dose range may be from about 0.01 mg to about 500 mg or any other dose, dependent upon the specific inhibitor, as is known in the art. In the case of a CETP inhibitor, the dose range may be from 5 about 0.01 mg to about 500 mg or any other dose, dependent upon the specific inhibitor, as is known in the art.

The active ingredients may also be administered by injection as a composition wherein, for example, saline, dextrose, or water may be used as a suitable carrier. A 10 suitable daily dose of each active therapeutic compound is one that achieves the same blood serum level as produced by oral administration as described above.

The therapeutic compounds may further be administered by any combination of oral/oral, oral/parenteral, or 15 parenteral/parenteral route.

Pharmaceutical compositions for use in the treatment methods of the present invention may be administered in oral form or by intravenous administration. Oral administration of the combination therapy is preferred. 20 Dosing for oral administration may be with a regimen calling for single daily dose, or for a single dose every other day, or for multiple, spaced doses throughout the day. The therapeutic compounds which make up the combination therapy may be administered simultaneously, 25 either in a combined dosage form or in separate dosage forms intended for substantially simultaneous oral administration. The therapeutic compounds which make up the combination therapy may also be administered sequentially, with either therapeutic compound being 30 administered by a regimen calling for two-step ingestion. Thus, a regimen may call for sequential administration of the therapeutic compounds with spaced-apart ingestion of the separate, active agents. The time period between the

-32-

multiple ingestion steps may range from a few minutes to several hours, depending upon the properties of each therapeutic compound such as potency, solubility, bioavailability, plasma half-life and kinetic profile of 5 the therapeutic compound, as well as depending upon the effect of food ingestion and the age and condition of the patient. Circadian variation of the target molecule concentration may also determine the optimal dose interval. The therapeutic compounds of the combined 10 therapy whether administered simultaneously, substantially simultaneously, or sequentially, may involve a regimen calling for administration of one therapeutic compound by oral route and another therapeutic compound by intravenous route. Whether the therapeutic compounds of the combined 15 therapy are administered by oral or intravenous route, separately or together, each such therapeutic compound will be contained in a suitable pharmaceutical formulation of pharmaceutically-acceptable excipients, diluents or other formulations components. Examples of suitable 20 pharmaceutically-acceptable formulations containing the therapeutic compounds for oral administration are given above.

Treatment Regimen

25 The dosage regimen to prevent, give relief from, or ameliorate a disease condition having hyperlipidemia as an element of the disease, e.g., atherosclerosis, or to protect against or treat further high cholesterol plasma or blood levels with the compounds and/or compositions of 30 the present invention is selected in accordance with a variety of factors. These include the type, age, weight, sex, diet, and medical condition of the patient, the severity of the disease, the route of administration,

-33-

pharmacological considerations such as the activity, efficacy, pharmacokinetics and toxicology profiles of the particular compound employed, whether a drug delivery system is utilized, and whether the compound is 5 administered as part of a drug combination. Thus, the dosage regimen actually employed may vary widely and therefore deviate from the preferred dosage regimen set forth above.

Initial treatment of a patient suffering from a 10 hyperlipidemic condition can begin with the dosages indicated above. Treatment should generally be continued as necessary over a period of several weeks to several months or years until the hyperlipidemic disease condition has been controlled or eliminated. Patients undergoing 15 treatment with the compounds or compositions disclosed herein can be routinely monitored by, for example, measuring serum LDL and total cholesterol levels by any of the methods well known in the art, to determine the effectiveness of the combination therapy. Continuous 20 analysis of such data permits modification of the treatment regimen during therapy so that optimal effective amounts of each type of therapeutic compound are administered at any point in time, and so that the duration of treatment can be determined as well. In this 25 way, the treatment regimen/dosing schedule can be rationally modified over the course of therapy so that the lowest amount of the therapeutic compounds which together exhibit satisfactory effectiveness is administered, and so that administration is continued only so long as is 30 necessary to successfully treat the hyperlipidemic condition.

A potential advantage of the combination therapy disclosed herein may be reduction of the amount of any

individual therapeutic compound, or all therapeutic compounds, effective in treating hyperlipidemic conditions such as atherosclerosis and hypercholesterolemia.

One of the several embodiments of the present invention comprises a combination therapy comprising the use of a first amount of an CETP inhibitor and a second amount of another cardiovascular therapeutic useful in the prophylaxis or treatment of hyperlipidemia, atherosclerosis, or hypercholesterolemia wherein said first and second amounts together comprise an anti-hyperlipidemic condition effective amount, an anti-atherosclerotic condition effective amount, or an anti-hypercholesterolemic condition effective amount of said compounds. For example one of the many embodiments of the present invention is a combination therapy comprising therapeutic dosages of a CETP inhibitor and an HMG CoA reductase inhibitor.

The embodiments of the present invention can comprise a combination therapy using two or more of the therapeutic compounds described or incorporated herein. The combination therapy can comprise two or more therapeutic compounds from different classes of chemistry, e.g., HMG CoA reductase inhibitors can be therapeutically combined with CETP inhibitors. Therapeutic combinations can comprise more than two therapeutic compounds. For example, the therapy can comprise the use of an IBAT inhibitor, a CETP inhibitor, and a HMG CoA reductase inhibitor. Alternatively, two or more therapeutic compounds from the same class of chemistry can comprise the therapy, e.g. a combination therapy comprising two or more HMG CoA reductase inhibitors or two or more CETP inhibitors.

-35-

A further embodiment of the instant invention comprises the use of any of the cardiovascular combination therapies described herein for the prophylaxis or treatment of hypercholesterolemia, atherosclerosis, or 5 hyperlipidemia.

The following non-limiting examples serve to illustrate various aspects of the present invention.

c. Examples

10

Table 3 illustrates examples of some combinations of the present invention wherein the combination comprises a first amount of a CETP inhibitor and a second amount of a HMG CoA reductase inhibitor, wherein said first and second 15 amounts together comprise an anti-hyperlipidemic condition effective amount, an anti-atherosclerotic condition effective amount, or an anti-hypercholesterolemic condition effective amount of said compounds.

20

Table 3.

Example Number	Component 1	Component 2
5751	C-1	mevastatin
5752	C-2	mevastatin
5753	C-3	mevastatin
5754	C-4	mevastatin
5755	C-5	mevastatin
5756	C-6	mevastatin
5757	C-7	mevastatin
5758	C-8	mevastatin
5759	C-9	mevastatin
5760	C-10	mevastatin
5761	C-11	mevastatin
5762	C-12	mevastatin
5763	C-13	mevastatin
5764	C-14	mevastatin

-36-

5765	C-15	mevastatin
5766	C-16	mevastatin
5767	C-17	mevastatin
5768	C-18	mevastatin
5769	C-19	mevastatin
5770	C-20	mevastatin
5771	C-1	lovastatin
5772	C-2	lovastatin
5773	C-3	lovastatin
5774	C-4	lovastatin
5775	C-5	lovastatin
5776	C-6	lovastatin
5777	C-7	lovastatin
5778	C-8	lovastatin
5779	C-9	lovastatin
5780	C-10	lovastatin
5781	C-11	lovastatin
5782	C-12	lovastatin
5783	C-13	lovastatin
5784	C-14	lovastatin
5785	C-15	lovastatin
5786	C-16	lovastatin
5787	C-17	lovastatin
5788	C-18	lovastatin
5789	C-19	lovastatin
5790	C-20	lovastatin
5791	C-1	simvastatin
5792	C-2	simvastatin
5793	C-3	simvastatin
5794	C-4	simvastatin
5795	C-5	simvastatin
5796	C-6	simvastatin
5797	C-7	simvastatin
5798	C-8	simvastatin
5799	C-9	simvastatin
5800	C-10	simvastatin
5801	C-11	simvastatin
5802	C-12	simvastatin
5803	C-13	simvastatin
5804	C-14	simvastatin

-37-

5805	C-15	simvastatin
5806	C-16	simvastatin
5807	C-17	simvastatin
5808	C-18	simvastatin
5809	C-19	simvastatin
5810	C-20	simvastatin
5811	C-1	pravastatin
5812	C-2	pravastatin
5813	C-3	pravastatin
5814	C-4	pravastatin
5815	C-5	pravastatin
5816	C-6	pravastatin
5817	C-7	pravastatin
5818	C-8	pravastatin
5819	C-9	pravastatin
5820	C-10	pravastatin
5821	C-11	pravastatin
5822	C-12	pravastatin
5823	C-13	pravastatin
5824	C-14	pravastatin
5825	C-15	pravastatin
5826	C-16	pravastatin
5827	C-17	pravastatin
5828	C-18	pravastatin
5829	C-19	pravastatin
5830	C-20	pravastatin
5831	C-1	fluvastatin
5832	C-2	fluvastatin
5833	C-3	fluvastatin
5834	C-4	fluvastatin
5835	C-5	fluvastatin
5836	C-6	fluvastatin
5837	C-7	fluvastatin
5838	C-8	fluvastatin
5839	C-9	fluvastatin
5840	C-10	fluvastatin
5841	C-11	fluvastatin
5842	C-12	fluvastatin
5843	C-13	fluvastatin
5844	C-14	fluvastatin

-38-

5845	C-15	fluvastatin
5846	C-16	fluvastatin
5847	C-17	fluvastatin
5848	C-18	fluvastatin
5849	C-19	fluvastatin
5850	C-20	fluvastatin
5851	C-1	atorvastatin
5852	C-2	atorvastatin
5853	C-3	atorvastatin
5854	C-4	atorvastatin
5855	C-5	atorvastatin
5856	C-6	atorvastatin
5857	C-7	atorvastatin
5858	C-8	atorvastatin
5859	C-9	atorvastatin
5860	C-10	atorvastatin
5861	C-11	atorvastatin
5862	C-12	atorvastatin
5863	C-13	atorvastatin
5864	C-14	atorvastatin
5865	C-15	atorvastatin
5866	C-16	atorvastatin
5867	C-17	atorvastatin
5868	C-18	atorvastatin
5869	C-19	atorvastatin
5870	C-20	atorvastatin

#### BIOLOGICAL ASSAYS

The utility of the combinations of the present invention can be shown by the following assays. These assays are performed *in vitro* and in animal models essentially using procedures recognized to show the utility of the present invention.

10 Measurement of Hepatic Cholesterol Concentration (HEPATIC CHOL)

-39-

Liver tissue is to be weighed and homogenized in chloroform:methanol (2:1). After homogenization and centrifugation the supernatant is separated and dried under nitrogen. The residue is to be dissolved in 5 isopropanol and the cholesterol content will be measured enzymatically, using a combination of cholesterol oxidase and peroxidase, as described by Allain, C. A. et al., Clin. Chem., 20, 470 (1974) (herein incorporated by reference).

10

Measurement of Hepatic HMG CoA-Reductase Activity (HMG COA)

Hepatic microsomes are to be prepared by homogenizing liver samples in a phosphate/sucrose buffer, followed by 15 centrifugal separation. The final pelleted material is resuspended in buffer and an aliquot will be assayed for HMG CoA reductase activity by incubating for 60 minutes at 37° C in the presence of <sup>14</sup>C-HMG-CoA (Dupont-NEN). The reaction is stopped by adding 6N HCl followed by 20 centrifugation. An aliquot of the supernatant is separated, by thin-layer chromatography, and the spot corresponding to the enzyme product is scraped off the plate, extracted and radioactivity is determined by scintillation counting. (Reference: Akerlund, J. and 25 Bjorkhem, I. (1990) *J. Lipid Res.* 31, 2159).

Determination of Serum Cholesterol (SER.CHOL, HDL-CHOL, TGI and VLDL + LDL)

Total serum cholesterol (SER.CHOL) are to be measured 30 enzymatically using a commercial kit from Wako Fine Chemicals (Richmond, VA); Cholesterol C11, Catalog No. 276-64909. HDL cholesterol (HDL-CHOL) will be assayed using this same kit after precipitation of VLDL and LDL

with Sigma Chemical Co. HDL Cholesterol reagent, Catalog No. 352-3 (dextran sulfate method). Total serum triglycerides (blanked) (TGI) will be assayed enzymatically with Sigma Chemical Co. GPO-Trinder, Catalog No. 337-B. VLDL and LDL (VLDL + LDL) cholesterol concentrations will be calculated as the difference between total and HDL cholesterol.

Measurement of Hepatic Cholesterol 7- $\alpha$ -Hydroxylase

10 Activity (7a-OHase)

Hepatic microsomes are to be prepared by homogenizing liver samples in a phosphate/sucrose buffer, followed by centrifugal separation. The final pelleted material is resuspended in buffer and an aliquot will be assayed for 15 cholesterol 7- $\alpha$ -hydroxylase activity by incubating for 5 minutes at 37° C in the presence of NADPH. Following extraction into petroleum ether, the organic solvent is evaporated and the residue is dissolved in acetonitrile/methanol. The enzymatic product will be separated by 20 injecting an aliquot of the extract onto a C<sub>18</sub> reversed phase HPLC column and quantitating the eluted material using UV detection at 240nm. (Reference: Horton, J. D., et al. (1994) *J. Clin. Invest.* 93, 2084).

25 Rat Gavage Assay

Male Wister rats (275-300g) are to be administered IBAT inhibitors using an oral gavage procedure. Drug or vehicle (0.2% TWEEN 80 in water) is administered once a day (9:00-10:0 a.m.) for 4 days at varying dosages in a 30 final volume of 2 mL per kilogram of body weight. (TWEEN 80 is a 20 molar polyethyleneoxide sorbitan monooleate surfactant manufactured by ICI Specialty Chemicals, Wilmington, Delaware, U.S.A.) Total fecal samples are

collected during the final 48 hours of the treatment period and analyzed for bile acid content using an enzymatic assay as described below. Compound efficacy will be determined by comparison of the increase in fecal 5 bile acid (FBA) concentration in treated rats to the mean FBA concentration of rats in the vehicle group.

Measurement of Rat Fecal Bile Acid Concentration (FBA)

Total fecal output from individually housed rats is 10 to be collected for 24 or 48 hours, dried under a stream of nitrogen, pulverized, mixed, and weighed. Approximately 0.1 gram is weighed out and extracted into an organic solvent (butanol/water). Following separation and drying, the residue is dissolved in methanol and the amount of 15 bile acid present will be measured enzymatically using the  $3\alpha$ -hydroxysteroid steroid dehydrogenase reaction with bile acids to reduce NAD. (see Mashige, F. et al. Clin. Chem., 27, 1352 (1981), herein incorporated by reference).

20 [ $^3$ H]Taurocholate Uptake in Rabbit Brush Border Membrane

Vesicles (BBMV)

Rabbit Ileal brush border membranes are to be prepared from frozen ileal mucosa by the calcium precipitation method describe by Malathi et al. 25 (Biochimica Biophysica Acta, 554, 259 (1979), herein incorporated by reference). The method for measuring taurocholate is essentially as described by Kramer et al. (Biochimica Biophysica Acta, 1111, 93 (1992), herein incorporated by reference) except the assay volume will be 30 200  $\mu$ l instead of 100  $\mu$ l. Briefly, at room temperature a 190  $\mu$ l solution containing 2 $\mu$ M [ $^3$ H]-taurocholate(0.75  $\mu$ Ci), 20 mM tris, 100 mM NaCl, 100 mM mannitol pH 7.4 is incubated for 5 sec with 10  $\mu$ l of brush border membrane

vesicles (60-120 µg protein). The incubation is initiated by the addition of the BBMV while vortexing and the reaction is to be stopped by the addition of 5 ml of ice cold buffer (20 mM Hepes-tris, 150 mM KCl) followed 5 immediately by filtration through a nylon filter (0.2 µm pore) and an additional 5 ml wash with stop buffer.

Dog Model for Evaluating Lipid Lowering Drugs

10 Male beagle dogs, obtained from a vendor such as Marshall farms and weighing 6-12 kg are fed once a day for two hours and given water ad libitum. Dogs may be randomly assigned to a dosing groups consisting of 6 to 12 dogs each, such as: vehicle, i.g.; 1mg/kg, i.g.; 2mg/kg, i.g.; 15 4 mg/kg, i.g.; 2 mg/kg, p.o. (powder in capsule). Intra-gastric dosing of a therapeutic material dissolved in aqueous solution (for example, 0.2% Tween 80 solution [polyoxyethylene mono-oleate, Sigma Chemical Co., St. Louis, MO]) may be done using a gavage tube. Prior to 20 initiating dosing, blood samples may be drawn from the cephalic vein in the morning before feeding in order to evaluate serum cholesterol (total and HDL) and triglycerides. For several consecutive days animals are dosed in the morning, prior to feeding. Animals are to be 25 allowed 2 hours to eat before any remaining food is removed. Feces are to be collected over a 2 day period at the end of the study and may be analyzed for bile acid or lipid content. Blood samples are also to be taken, at the end of the treatment period, for comparison with pre-study 30 serum lipid levels. Statistical significance will be determined using the standard student's T-test with p<.05.

**Dog Serum Lipid Measurement**

Blood is to be collected from the cephalic vein of fasted dogs in serum separator tubes (Vacutainer SST, 5 Becton Dickinson and Co., Franklin Lakes, NJ). The blood is centrifuged at 2000 rpm for 20 minutes and the serum decanted.

Total cholesterol may be measured in a 96 well format using a Wako enzymatic diagnostic kit (Cholesterol CII) 10 (Wako Chemicals, Richmond, VA), utilizing the cholesterol oxidase reaction to produce hydrogen peroxide which is measured colorimetrically. A standard curve from 0.5 to 10 µg cholesterol is to be prepared in the first 2 columns of the plate. The serum samples (20-40 µl, depending on 15 the expected lipid concentration) or known serum control samples are added to separate wells in duplicate. Water is added to bring the volume to 100 µl in each well. A 100 µl aliquot of color reagent is added to each well and the plates will be read at 500 nm after a 15 minute 20 incubation at 37 degrees centigrade.

HDL cholesterol may be assayed using Sigma kit No. 352-3 (Sigma Chemical Co., St. Louis, MO) which utilizes dextran sulfate and Mg ions to selectively precipitate LDL and VLDL. A volume of 150 µl of each serum sample is to 25 be added to individual microfuge tubes, followed by 15 µl of HDL cholesterol reagent (Sigma 352-3). Samples are to be mixed and centrifuged at 5000 rpm for 5 minutes. A 50 µl aliquot of the supernatant is to be then mixed with 200 µl of saline and assayed using the same procedure as for 30 total cholesterol measurement.

Triglycerides are to be measured using Sigma kit No. 337 in a 96 well plate format. This procedure will

measure glycerol, following its release by reaction of triglycerides with lipoprotein lipase. Standard solutions of glycerol (Sigma 339-11) ranging from 1 to 24 µg are to be used to generate the standard curve. Serum samples 5 (20-40 µl, depending on the expected lipid concentration) are added to wells in duplicate. Water is added to bring the volume to 100 µl in each well and 100 µl of color reagent was also added to each well. After mixing and a 15 minute incubation, the plates will be read at 540 nm 10 and the triglyceride values calculated from the standard curve. A replicate plate is also to be run using a blank enzyme reagent to correct for any endogenous glycerol in the serum samples.

15       **Dog Fecal Bile Acid Measurement**

Fecal samples may be collected to determine the fecal bile acid (FBA) concentration for each animal. Fecal collections may be made during the final 48 hours of the study, for two consecutive 24 hour periods between 9:00 am 20 and 10:00 am each day, prior to dosing and feeding. The separate two day collections from each animal are to be weighed, combined and homogenized with distilled water in a processor (Cuisinart) to generate a homogeneous slurry. About 1.4 g of the homogenate is to be extracted in a 25 final concentration of 50% tertiary butanol/distilled water (2:0.6) for 45 minutes in a 37°C water bath and centrifuged for 13 minutes at 2000 x g. The concentration of bile acids (mmoles/day) may be determined using a 96-well enzymatic assay system (1,2). A 20 µl aliquot of the 30 fecal extract is to be added to two sets each of triplicate wells in a 96-well assay plate. A standardized sodium taurocholate solution and a standardized fecal extract solution (previously made from pooled samples and

-45-

characterized for its bile acid concentration) will also be analyzed for assay quality control. Twenty-microliter aliquots of sodium taurocholate, serially diluted to generate a standard curve are similarly to be added to two sets of triplicate wells. A 230  $\mu$ l reaction mixture containing 1M hydrazine hydrate, 0.1 M pyrophosphate and 0.46 mg/ml NAD is to be added to each well. A 50  $\mu$ l aliquot of 3 $\alpha$ -hydroxysteroid dehydrogenase enzyme (HSD; 0.8 units/ml), or assay buffer (0.1 M sodium pyrophosphate) are then added to one of the two sets of triplicates. All reagents may be obtained from Sigma Chemical Co., St. Louis, MO. Following 60 minutes of incubation at room temperature, the optical density at 340nm will be measured and the mean of each set of triplicate samples will be calculated. The difference in optical density  $\pm$  HSD enzyme is to be used to determine the bile acid concentration (mM) of each sample based on the sodium taurocholate standard curve. The bile acid concentration of the extract, the weight of the fecal homogenate (grams) and the body weight of the animal are to be used to calculate the corresponding FBA concentration in mmoles/kg/day for each animal. The mean FBA concentration (mmoles/kg/day) of the vehicle group is to be subtracted from the FBA concentration of each treatment group to determine the increase (delta value) in FBA concentration as a result of the treatment.

CETP ACTIVITY ASSAY IN HUMAN PLASMA (Tritiated  
30 cholesteryl ester)  
Blood is to be obtained from healthy volunteers. Blood is collected in tubes containing EDTA (EDTA plasma pool). The EDTA human plasma pool previously stored at -

20°C, is to be thawed at room temperature, and centrifuged for 5 minutes to remove any particulate matter. Tritiated HDL, radiolabeled in the cholesteryl ester moiety ([<sup>3</sup>H]CE-HDL) as described by Morton and Zilversmit (J. Biol. 5 Chem., 256, 11992-95 (1981)), is to be added to the plasma to a final concentration of (25 µg/ml cholesterol). Inhibitor compounds are to be added to the plasma as follows: Equal volumes of the plasma containing the [<sup>3</sup>H]CE-HDL (396 µl) are added by pipette into micro tubes 10 (Titertube®, Bio-Rad laboratories, Hercules, CA). Compounds, usually dissolved as 20-50 mM stock solutions in DMSO, are to be serially diluted in DMSO (or an alternative solvent in some cases, such as dimethylformamide or ethanol). Four µl of each of the 15 serial dilutions of inhibitor compounds or DMSO alone are then added to each of the plasma tubes. The tubes are immediately mixed. Triplicate aliquots (100 µl) from each plasma tube are then transferred to wells of 96-well round-bottomed polystyrene microtiter plates (Corning, 20 Corning, NY). Plates are sealed with plastic film and incubated at 37°C for 4 hours. Test wells are to contain plasma with dilutions of inhibitor compounds. Control wells are to contain plasma with DMSO alone. Blank wells are to contain plasma with DMSO alone that are left in the 25 micro tubes at 4°C for the 4 hour incubation and are added to the microtiter wells at the end of the incubation period. VLDL and LDL are precipitated by the addition of 10 µl of precipitating reagent (1% (w/v) dextran sulfate (Dextralip50)/0.5 M magnesium chloride, pH 7.4) to all 30 wells. The wells are mixed on a plate mixer and then incubated at ambient temperature for 10 min. The plates are then centrifuged at 1000 x g for 30 min at 10°C. The

-47-

supernatants (50  $\mu$ l) from each well are then transferred to Picoplate<sup>TM</sup> 96 plate wells (Packard, Meriden, CT) containing 250:1 Microscint<sup>TM</sup>-40 (Packard, Meriden, CT). The plates are heat-sealed (TopSeal<sup>TM</sup>-P, Packard, Meriden, 5 CT) according to the manufacturer's directions and mixed for 30 min. Radioactivity will be measured on a microplate scintillation counter (TopCount, Packard, Meriden, CT). IC<sub>50</sub> values will be determined as the concentration of inhibitor compound inhibiting transfer of 10 [<sup>3</sup>H]CE from the supernatant [<sup>3</sup>H]CE-HDL to the precipitated VLDL and LDL by 50% compared to the transfer obtained in the control wells. The maximum percentage transfer (in the control wells) will be determined using the following equation:

15

$$\% \text{ Transfer} = \frac{[\text{dpm}_{\text{blank}} - \text{dpm}_{\text{control}}] \times 100}{\text{dpm}_{\text{blank}}}$$

The percentage of control transfer determined in the wells containing inhibitor compounds is determined as follows:

20

$$\% \text{ Control} = \frac{[\text{dpm}_{\text{blank}} - \text{dpm}_{\text{test}}] \times 100}{\text{dpm}_{\text{blank}} - \text{dpm}_{\text{control}}}$$

IC<sub>50</sub> values will be calculated from plots of % control versus concentration of inhibitor compound.

25

#### CETP Activity In Vitro

The ability of compounds to inhibit CETP activity are assessed using an *in vitro* assay that measures the rate of transfer of radiolabeled cholestryly ester ([<sup>3</sup>H]CE) from 30 HDL donor particles to LDL acceptor particles. Details of

the assay are provided by Glenn et al. (Glenn and Melton, "Quantification of Cholesteryl Ester Transfer Protein (CETP): A) CETP Activity and B) Immunochemical Assay of CETP Protein," Meth. Enzymol., 263, 339-351 (1996)). CETP can be obtained from the serum-free conditioned medium of CHO cells transfected with a cDNA for CETP (Wang, S. et al. J. Biol. Chem. 267, 17487-17490 (1992)). To measure CETP activity, [<sup>3</sup>H]CE-labeled HDL, LDL, CETP and assay buffer (50 mM tris(hydroxymethyl)aminomethane, pH 7.4; 150 mM sodium chloride; 2 mM ethylenediamine-tetraacetic acid; 1% bovine serum albumin) are incubated in a volume of 200 µl, for 2 hours at 37°C in 96 well plates. LDL is differentially precipitated by the addition of 50 µl of 1% (w/v) dextran sulfate/0.5 M magnesium chloride, mixed by vortex, and incubated at room temperature for 10 minutes. The solution (200 µl) is transferred to a filter plate (Millipore). After filtration, the radioactivity present in the precipitated LDL is measured by liquid scintillation counting. Correction for non-specific transfer or precipitation is made by including samples that do not contain CETP. The rate of [<sup>3</sup>H]CE transfer using this assay is linear with respect to time and CETP concentration, up to 25-30% of [<sup>3</sup>H]CE transferred.

The potency of test compounds can be determined by performing the above described assay in the presence of varying concentrations of the test compounds and determining the concentration required for 50% inhibition of transfer of [<sup>3</sup>H]CE from HDL to LDL. This value is defined as the IC<sub>50</sub>. The IC<sub>50</sub> values determined from this assay will be accurate when the IC<sub>50</sub> is greater than 10 nM. In the case where compounds have greater inhibitory potency, accurate measurements of IC<sub>50</sub> may be determined

using longer incubation times (up to 18 hours) and lower final concentrations of CETP (< 50 nM).

##### 5 Inhibition of CETP Activity In Vivo.

Inhibition of CETP activity by a test compound can be determined by administering the compound to an animal by intravenous injection or oral gavage, measuring the amount of transfer of tritium-labeled cholestryl ester ( $[^3\text{H}]CE$ ) from HDL to VLDL and LDL particles, and comparing this amount of transfer with the amount of transfer observed in control animals.

Male golden Syrian hamsters are to be maintained on a diet of chow containing 0.24% cholesterol for at least two weeks prior to the study. For animals receiving intravenous dosing, immediately before the experiment, animals are anesthetized with pentobarbital. Anesthesia is maintained throughout the experiment. In-dwelling catheters are to be inserted into the jugular vein and carotid artery. At the start of the experiment all animals will receive 0.2 ml of a solution containing  $[^3\text{H}]CE$ -HDL into the jugular vein.  $[^3\text{H}]CE$ -HDL is a preparation of human HDL containing tritium-labeled cholestryl ester, and is prepared according to the method of Glenn et al. (Meth. Enzymol., 263, 339-351 (1996)). Test compound is dissolved as a 80 mM stock solution in vehicle (2% ethanol: 98% PEG 400, Sigma Chemical Company, St. Louis, Missouri, USA) and administered either by bolus injection or by continuous infusion. Two minutes after the  $[^3\text{H}]CE$ -HDL dose is administered, animals are to receive 0.1 ml of the test solution injected into the jugular vein. Control animals are to receive 0.1 ml of the intravenous vehicle solution without test compound.

After 5 minutes, the first blood samples (0.5 ml) are taken from the carotid artery and collected in standard microtainer tubes containing ethylenediamine tetraacetic acid. Saline (0.5 ml) is injected to flush the catheter 5 and replace blood volume. Subsequent blood samples are to be taken at two hours and four hours by the same method. Blood samples are mixed well and kept on ice until the completion of the experiment. Plasma is obtained by centrifugation of the blood samples at 4° C. The plasma 10 (50 µl) is treated with 5 µl of precipitating reagent (dextran sulfate, 10 g/l; 0.5 M magnesium chloride) to remove VLDL/LDL. After centrifugation, the resulting supernatant (25 µl) containing the HDL will be analyzed for radioactivity using a liquid scintillation counter.

15 The percentage [<sup>3</sup>H]CE transferred from HDL to LDL and VLDL (% transfer) will be calculated based on the total radioactivity in equivalent plasma samples before precipitation. Typically, the amount of transfer from HDL to LDL and VLDL in control animals will be 20% to 35% 20 after 4 hours.

Alternatively, conscious, non-anesthetized animals can receive an oral gavage dose of test compound as a suspension in 0.1% methyl cellulose in water. At a time determined for each compound at which plasma levels of the 25 test substance reach their peak ( $C_{max}$ ) after oral dosing, the animals are to be anesthetized with pentobarbital and then dosed with 0.2 ml of a solution containing [<sup>3</sup>H]CE-HDL into the jugular vein as described above. Control animals are to receive 0.25 ml of the vehicle solution without 30 test compound by oral gavage. After 4 hours, the animals are to be sacrificed, blood samples are collected, and the percentage [<sup>3</sup>H]CE transferred from HDL to LDL and VLDL (% transfer) is assayed as described above.

Alternatively, inhibition of CETP activity by a test compound can be determined by administering the compound to mice that have been selected for expression of human CETP (hCETP) by transgenic manipulation (hCETP mice).

- 5 Test compounds can be administered by intravenous injection, or oral gavage and the amount of transfer of tritium-labeled cholestryl ester ( $[^3\text{H}]CE$ ) from HDL to VLDL and LDL particles is determined, and compared to the amount of transfer observed in control animals. C57Bl/6  
10 mice that are homozygous for the hCETP gene are to be maintained on a high fat chow diet, such as TD 88051, as described by Nishina et al. (J Lipid Res., 31, 859-869 (1990)) for at least two weeks prior to the study. Mice are to receive an oral gavage dose of test compound as a  
15 suspension in 0.1% methyl cellulose in water or an intravenous bolus injection of test compound in 10% ethanol and 90% polyethylene glycol. Control animals are to receive the vehicle solution without test compound by oral gavage or by an intravenous bolus injection. At the  
20 start of the experiment all animals will receive 0.05 ml of a solution containing  $[^3\text{H}]CE$ -HDL into the tail vein.  
 $[^3\text{H}]CE$ -HDL will be a preparation of human HDL containing tritium-labeled cholestryl ester, and is prepared according to the method of Glenn et al. (Meth. Enzymol.,  
25 263, 339-351 (1996)). After 30 minutes, the animals are exsanguinated and blood collected in standard microtainer tubes containing ethylenediamine tetraacetic acid. Blood samples are mixed well and kept on ice until the completion of the experiment. Plasma will be obtained by  
30 centrifugation of the blood samples at 4°C. The plasma is separated and analyzed by gel filtration chromatography and the relative proportion of  $[^3\text{H}]CE$  in the VLDL, LDL and HDL regions will be determined.

The percentage [<sup>3</sup>H]CE transferred from HDL to LDL and VLDL (% transfer) will be calculated based on the total radioactivity in equivalent plasma samples before precipitation. Typically, the amount of transfer from HDL 5 to LDL and VLDL in control animals will be 20% to 35% after 30 min.

Plasma Lipids Assay in Rabbits

Plasma lipids can be assayed using standard methods 10 as reported by J.R. Schuh et al., J. Clin. Invest., 91, 1453-1458 (1993), herein incorporated by reference. Groups of male, New Zealand white rabbits are placed on a standard diet (100g/day) supplemented with 0.3% cholesterol and 2% corn oil (Zeigler Brothers, Inc., 15 Gardners, PA). Water is available ad lib. Groups of control and treated animals are killed after 1 and 3 months of treatment. Tissues are removed for characterization of atherosclerotic lesions. Blood samples are taken for determination of plasma lipid 20 concentrations.

**Plasma Lipids**

Plasma for lipid analysis is obtained by withdrawing blood from the ear vein into EDTA-containing tubes 25 (Vacutainer; Becton Dickenson & Co., Rutherford, NJ), followed by centrifugal separation of the cells. Total cholesterol was determined enzymatically, using the cholesterol oxidase reaction (C.A. Allain et al., Clin. Chem., 20, 470-475 (1974), herein incorporated by reference). HDL cholesterol was also measured 30 enzymatically, after selective precipitation of LDL and VLDL by dextran sulfate with magnesium (G.R. Warnick et al., Clin. Chem., 28, 1379-1388 (1982), herein

incorporated by reference). Plasma triglyceride levels are determined by measuring the amount of glycerol released by lipoprotein lipase through an enzyme-linked assay (G. Bucolo et al., Clin. Chem., 19, 476-482 (1973), 5 herein incorporated by reference).

#### Atherosclerosis

Animals are killed by pentobarbital injection. Thoracic aortas are rapidly removed, immersion fixed in 10 10% neutral buffered formalin, and stained with oil red O (0.3%). After a single longitudinal incision along the wall opposite the arterial ostia, the vessels are pinned open for evaluation of the plaque area. The percent 15 plaque coverage is determined from the values for the total area examined and the stained area, by threshold analysis using a true color image analyzer (Videometric 150; American Innovision, Inc., San Diego, CA) interfaced to a color camera (Toshiba 3CCD) mounted on a dissecting microscope. Tissue cholesterol will be measured 20 enzymatically as described, after extraction with a chloroform/methanol mixture (2:1) according to the method of Folch et al. (J. Biol. Chem., 226, 497-509 (1957), herein incorporated by reference).

#### 25 In Vitro Vascular Response

The abdominal aortas are rapidly excised, after injection of sodium pentobarbital, and placed in oxygenated Krebs-bicarbonate buffer. After removal of perivascular tissue, 3-mm ring segments are cut, placed in a 37°C muscle bath 30 containing Krebs-bicarbonate solution, and suspended between two stainless steel wires, one of which is attached to a force transducer (Grass Instrument Co.,

Quincy, MA). Force changes in response to angiotensin II added to the bath will be recorded on a chart recorder.

5       The examples herein can be performed by substituting the generically or specifically described therapeutic compounds or inert ingredients for those used in the preceding examples.

The invention being thus described, it is apparent  
10 that the same can be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the present invention, and all such modifications and equivalents as would be obvious to one skilled in the art are intended to be included within the scope of the  
15 following claims.

CLAIMS

What is claimed is:

1. A therapeutic combination comprising a first amount  
5 of an HMG CoA reductase inhibiting compound and a second amount of a cholesteryl ester transfer protein inhibiting compound wherein the first amount and the second amount together comprise an anti-hyperlipidemic condition effective amount, an anti-atherosclerotic condition effective amount, or 10 an anti-hypercholesterolemic condition effective amount of the compounds.
2. The therapeutic combination of claim 1 wherein the  
15 HMG CoA reductase inhibiting compound comprises mevastatin.
3. The therapeutic combination of claim 1 wherein the  
20 HMG CoA reductase inhibiting compound comprises lovastatin.
4. The therapeutic combination of claim 1 wherein the  
25 HMG CoA reductase inhibiting compound comprises simvastatin.
5. The therapeutic combination of claim 1 wherein the  
HMG CoA reductase inhibiting compound comprises pravastatin.
- 30 6. The therapeutic combination of claim 1 wherein the HMG CoA reductase inhibiting compound comprises fluvastatin.

-56-

combination of claim 1 wherein the  
se inhibiting compound comprises

combination of claim 1 wherein the  
prises a composition comprising the  
se inhibiting compound and the  
ter transfer protein inhibiting

the prophylaxis or treatment of a  
condition comprising administering  
in need thereof a combination in unit  
erein the combination comprises a  
an HMG CoA reductase inhibiting  
second amount of a cholestryl ester  
n inhibiting compound wherein the  
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hi-hyperlipidemic condition effective  
compounds.

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erein the combination comprises a  
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second amount of a cholestryl ester  
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## INTERNATIONAL SEARCH REPORT

Int. Application No

PCT/US 99/27943

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K45/06 A61P9/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 818 197 A (BAYER) 14 January 1998 (1998-01-14) cited in the application claims 1,8 page 14 page 16, line 9-14 page 18, line 37-52	1,3-11

 Further documents are listed in the continuation of box C. Patent family members are listed in annex.

## \* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

16 May 2000

Date of mailing of the international search report

23/05/2000

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# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 99/27943

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP 818197	A 14-01-1998	DE AU BG BR CA CN CZ HR HU JP NO PL SG SK US	19627431 A 715101 B 101748 A 9703890 A 2209825 A 1174196 A 9702144 A 970333 A 9701157 A 10167967 A 973143 A 320953 A 46781 A 92597 A 5932587 A	15-01-1998 13-01-2000 30-04-1998 03-11-1998 08-01-1998 25-02-1998 14-01-1998 30-04-1998 30-03-1998 23-06-1998 09-01-1998 19-01-1998 20-02-1998 06-05-1998 03-08-1999